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Title:

Glucose metabolism disorders in children with refractory nephrotic syndrome

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Abstract

Background: Patients with refractory nephrotic syndrome (NS) are at high risk of medication-induced glucose metabolism disorders, because of their long-term use of diabetogenic medications, particularly glucocorticoids and calcineurin inhibitors (CNIs). However, there have been no comprehensive evaluations of glucose metabolism disorders in pediatric patients with refractory NS. Moreover, glucocorticoids and CNIs could not be discontinued in these patients until the effectiveness of rituximab on refractory NS was shown and, therefore, there has been limited opportunity to evaluate glucose metabolism disorders after discontinuation of these medications.

Methods: Consecutive pediatric patients who started rituximab treatment for refractory NS were enrolled. Their glucose metabolism conditions were evaluated using the oral glucose tolerance tests (OGTT) and HbA1c levels at the initiation of rituximab treatment. Patients with glucose metabolism disorders at the first evaluation were reevaluated after approximately 2 years.

Results: Overall, 57% (20/35) of study patients had glucose metabolism disorders, and 40% (8/20) of these patients were detected only by their 2-hour OGTT blood glucose levels and not by their fasting blood glucose or HbA1c levels. Non-obese/non-overweight patients had significantly more glucose metabolism disorders than obese/overweight patients (p=0.019). In addition, glucose metabolism

disorders in 71% (10/14) of patients persisted after the discontinuation of glucocorticoids and CNIs.

Conclusions: Whether the patient is obese/overweight or not, patients with refractory NS are at high risk of developing glucose metabolism disorders, even in childhood. Non-obese/non-overweight patients who are at high risk of diabetes need extra vigilance.

Keywords: glucose metabolism disorders, oral glucose tolerance test, nephrotic syndrome, glucocorticoids, calcineurin inhibitors

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Introduction

Idiopathic nephrotic syndrome (NS) is the most common chronic glomerular disease in children and occurs in ≥2 children per 100,000.[1] A total of 20%–30% of children with this syndrome experience frequent relapse or steroid dependence during immunosuppressive therapies or immediately after their discontinuation.[2] These patients are exposed to long-term and often high-dose diabetogenic medications, particularly glucocorticoids and calcineurin inhibitors (CNIs).[3] Prediabetes, which is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold, is associated with an enhanced risk for development of type 2 diabetes in adults.[4] Therefore, even if they have not yet developed diabetes, glucose metabolism disorders should not be overlooked. However, specific guidelines are not available for screening patients in the pediatric age group treated with these diabetogenic medications.

In general, hyperglycemia induced by glucocorticoids or CNIs improves with dose reduction and reverses with discontinuation of medications; however, some patients may develop persistent glucose metabolism disorders.[5] In fact, we have previously reported that glucose metabolism disorders in patients with refractory NS might not always be reversible even after discontinuation of glucocorticoids and CNIs.[6] Moreover, some patients with refractory NS cannot discontinue their medications in adulthood,[7-11]

indicating that their diabetogenic risk lasts for a prolonged time after their childhood.

There have been no reports on the comprehensive evaluation of glucose metabolism disorders in these high-risk pediatric patients, especially based on rigorous definitions and using the oral glucose tolerance tests (OGTT). Moreover, we could not evaluate their glucose metabolism disorders after the discontinuation of glucocorticoids and CNIs because these medications could not be discontinued until the effectiveness of rituximab on refractory NS was shown. In this study, we comprehensively aimed to perform a cross-sectional evaluation on glucose metabolism disorders in pediatric patients with refractory NS who started rituximab treatment. In addition, patients with glucose metabolism disorders at initial evaluation were reevaluated after discontinuation of some diabetogenic medications. This is the first report about glucose metabolism disorders in patients with refractory NS who have discontinued glucocorticoids and CNIs.

Materials and Methods

Study population

We enrolled 35 consecutive pediatric patients who started rituximab treatment at Hokkaido University hospitals, Hokkaido, Japan, between January 2015 and July 2018. All of them were diagnosed as having

refractory NS who developed frequent relapse NS (FRNS) or steroid-dependent NS (SDNS) during CNI administration or after discontinuation of CNI. The definitions of FRNS and SDNS were those mentioned in the International Study of Kidney Disease in Children.[12]

Data collection

Anthropometric measurement, measurement of hemoglobin A1c (HbA1c) levels, and OGTT were performed during initial rituximab administration. Body mass index (BMI) was calculated with the standard formula: BMI = weight (kg)/height^2 (m^2). Overweight was defined as having a BMI at or above the 85th percentile and below the 95th percentile for children and teens of the same age and sex.[13] Obesity was defined as having a BMI at or above the 95th percentile for children and teens of the same age and sex. Family history of diabetes was defined as positive if at least one first-degree relative had diabetes or prediabetes. A standard OGTT was performed for all patients according to the recommendations of World Health Organization.[14] After overnight fasting, patients were invited to drink a solution with 1.75 g of glucose per kilogram of body weight to a maximum of 75 g. Blood samples were collected at 0, 30, 60, and 120 minutes after drinking the solution, and plasma glucose and insulin concentrations were measured. In addition, patients with glucose metabolism disorders at the first evaluation were reevaluated after approximately 2 years. The diagnoses of diabetes and prediabetes were

based on American Diabetes Association (ADA) criteria, which are also stated in the International Society for Pediatric and Adolescent Diabetes clinical practice consensus guidelines 2014 compendium.[15, 16] Diabetes was diagnosed when fasting plasma glucose (FPG) level was ≧126 mg/dL or 2-hour blood glucose level was ≧200 mg/dL during OGTT or HbA1c level was ≧6.5% or random blood glucose level was ≧200 mg/dL. Based on the results of OGTT, patients were classified as normal glucose tolerant (NGT) if FPG level was <100 mg/dL and 2-hour post-load glucose was <140 mg/dL; as having impaired fasting glucose (IFG) if FPG level was 100–125 mg/dL; as having impaired glucose tolerance (IGT) if 2-hour post-load glucose was 140–199 mg/dL. Patients with prediabetes are defined by the presence of IFG and/or IGT and/or HbA1c levels of 5.7%–6.4%. To assess insulin resistance, we used homeostatic model assessment of insulin resistance (HOMA-IR = fasting serum insulin level × fasting blood glucose/405).[17] To assess beta-cell secretion, we used the insulinogenic index and the ratio of area under the insulin curve to the area under the glucose curve ($AUC_{ins/glu}$). Insulinogenic index was calculated as the ratio of the increment in the plasma insulin level to that in the plasma glucose level during the first 30 minutes after the ingestion of glucose. Area under the curve was calculated by the trapezoid method.

Therapeutic protocol for NS

Rituximab administration was performed 2 ~ 4 times during the study period. As post-rituximab therapy, mizoribine was given orally twice a week in a dose of 500mg on day 1 and 550mg on day 2. Tapering of CNI began at the time of the first dose of rituximab, and the CNI was discontinued one week later. Tapering of PSL began at 2weeks after the first dose of rituximab with discontinuation 2 to 3 months later. When patients had a relapse during the study period, they received 60mg/㎡ oral PSL (maximum 60mg) in three divided doses until proteinuria disappeared for 3 consecutive days. Thereafter, PSL was switched to alternate days, and the dose was gradually tapered every 2 weeks over a 2-month period.

Statistical analysis

Differences in demographic and metabolism characteristics between patients classified as obesity/overweight and those not were examined using a Wilcoxon signed-rank test. Fisher's exact tests were used for categorical variables. The Wilcoxon rank-sum test was used to compare glycometabolic characteristics by OGTT between the first and follow-up evaluation. Significance was set at $P<0.05$. All analyses were performed with JMP version 11.0 (SAS Institute Japan, Tokyo, Japan).

Results

Diagnoses

Table 1 shows background characteristics at the first evaluation. A total of 34 of 35 patients had received CNI treatment at the first evaluation, and among them only one patient used tacrolimus instead of cyclosporine A. Four patients had first-degree family history; one mother with diabetes was diagnosed during her pregnancy and the disease persisted also after the pregnancy, and three parents with prediabetes were diagnosed based on results of the periodical medical examination at their company. We diagnosed one severely obese patient as having diabetes without OGTT because her HbA1c level was 10% and random blood glucose level was >200 mg/dL. Another patient who received steroid pulse therapy just before the first evaluation and had an HbA1c level of 6.0% was diagnosed as having prediabetes without OGTT. As a result, 57% (20/35) of pediatric patients with refractory NS had glucose metabolism disorders. There were 2 patients with diabetes and 18 patients with prediabetes. Nine patients showed abnormal HbA1c levels. Among them, 8 patients had abnormal OGTT values, and one patient had normal OGTT values. At the first OGTT, one patient was found to have diabetes; 4 patients, IFG+IGT; and 12 patients, IGT. Among 20 patients with glucose metabolism disorders, 8 patients (40%) were detected only by their 2-hour OGTT blood glucose levels and not by their fasting blood glucose or HbA1c levels. Although one patient had used growth hormone for short stature, he had normal glucose metabolism.

Obesity and Overweight

Five of 15 (33%) obese/overweight patients had glucose metabolism disorders. On the contrary, 15 of 20 (75%) non-obese/non-overweight patients had glucose metabolism disorders. Sex, disease duration, prednisolone (PSL) dose, and CNI treatment duration were not significantly different between obese/overweight patients and non-obese/non-overweight patients. On the contrary, non-obese/non-overweight patients had glucose metabolism disorders significantly more frequently than obese/overweight patients. Obese/overweight patients showed a significantly high level of HOMA-IR, insulinogenic index, and $AUC_{ins/glu}$. All patients with first-degree family history were non-obese/non-overweight (Table 2).

Follow-up evaluation

All but one patient had no specific intervention, including pharmaceutical intervention or specific dietary strategies, although we requested that all patients pay attention to their diet and exercise. Only one patient (Patient 1) who was diagnosed as having diabetes received metformin temporarily. Fourteen patients were reevaluated approximately 2 years after the first evaluation. The remaining 6 patients were lost to follow-up on account of transition or changing hospitals. At the follow-up evaluation, 10 patients still had prediabetes, even though they had a median 15.5 months washout period of PSL and almost 2 years'

washout periods of CNI. Among them, two patients (Patient 7 & 8) showed an increase in their BMI, and one patient (Patient 1) showed a decrease in her BMI but still remained severely obese. One patient (Patient 7) had no washout period of PSL but had a 2-years washout period of CNI. Although there was no relapse without PSL and CNI for more than one and a half years after rituximab treatment, he unfortunately experienced a relapse 3 months before the follow-up evaluation; consequently, PSL was discontinued just before the follow-up evaluation. One patient in the first evaluation (Patient 8) and two patients in the follow-up evaluation (Patient 9 & 10) had abnormal HbA1c levels and were NGT. It is difficult to make a judgment whether these cases truly had glucose metabolism abnormality. The other 5 patients (Patients 2-6) showed similar tendency: non-obese/non-overweight both at the first and the follow-up evaluation, enough PSL and CNI washout period, and no discrepancy between HbA1c and OGTT. They had only 2-hour blood glucose abnormality at the first OGTT, but in 4 of them fasting glucose abnormality appeared at the follow-up OGTT after the discontinuation of PSL and CNI. Two non-obese/non-overweight patients (Patient 2 & 7) showed a remarkably decreasing insulinogenic index (<0.4) at the follow-up evaluation (Table 3). In these two patients, islet cell antibodies were measured, but not detected. Insulinogenic index and $AUC_{ins/glu}$ at the follow-up evaluation were significantly lower than at the first evaluation (p=0.001). Fasting blood glucose at the follow-up evaluation was significantly

higher than at the first evaluation (p=0.025) (Table 4). This differentiation was more obvious in non-obese/non-overweight patients, excluding one patient without PSL administration at the first evaluation (Fig. 1).

Discussion

This study is a cross-sectional evaluation of glucose metabolism parameters in pediatric patients with refractory NS who started rituximab treatment in combination with follow-up evaluation after rituximab treatment. Overall, 57% of pediatric patients with refractory NS had glucose metabolism disorders. Contrary to a general perception that obese/overweight patients have a higher risk of type 2 diabetes mellitus than non-obese/non-overweight patients, in this study's population non-obese/non-overweight patients had glucose metabolism disorders more frequently than obese/overweight patients. In addition, glucose metabolism disorders in 71% (10/14) of patients, who had glucose metabolism disorders at the first evaluation and who were able to be reevaluated, persisted after the discontinuation of PSL and CNI. Previous studies have demonstrated some form of glucose metabolism disorders in adults with chronic steroid use for various conditions, with prevalence between 0.4% and 54%.[18, 19] The prevalence has varied across studies and has been difficult to establish because of the variation in diagnostic criteria, length of follow-up, type of immunosuppression, and racial difference. There are few reports about

medication-induced diabetes in pediatric patients with NS, although there are some reports on organ transplantation or hematological malignancy.[20-24] The immunosuppressive regimens of refractory NS in our study were rather similar to kidney transplantation. Tillmann et al. have shown that 30.5% of consecutive renal transplantation patients were diagnosed as having prediabetes.[25] In any case, the prevalence of glucose metabolism disorders in our study population seemed to be high.

There are only limited data on risk factors for steroid-induced diabetes, and the existing evidence in both children and adult is conflicting.[5] In this report, non-obese/non-overweight patients had significantly developed glucose metabolism disorders more frequently than obese/overweight patients. It has been reported that 10%–56% of obese children without any underlying disease had glucose metabolism disorders.[26, 27] In this report, 33% of obese/overweight patients had developed glucose metabolism disorders, which was not higher than the rate of obese children without underlying disease cited above. On the contrary, it has been reported that 5.4% of healthy children (non-obese/non-overweight) had even developed glucose metabolism disorders,[4] although surprisingly 75% of non-obese/non-overweight patients had developed glucose metabolism disorders in this report. It has been reported that the association of obesity with glucose metabolism disorders is not as strong in children and adolescents as it is in adults.[28] Moreover, especially Japanese children with type 2 diabetes are likely to be thinner than

Caucasian children and approximately 10%-15% of Japanese children with type2 diabetes exhibit normal weight with mild insulin resistance and substantial insulin secretion failure.[29, 30] It is popularly thought that basic genetic defect of the β cell is required for the development of type 2 diabetes, and insulin resistance is merely an acquired stimulus.[31] In the face of severe peripheral insulin resistance, patients who cannot maintain adequate β-cell secretion, probably because of genetically determined factors, easily develop glucose metabolism disorders.[32, 33] In this study, we speculate that glucose metabolism disorders might be actualized in patients who have fragility of the β-cell function genetically and probably have normal weight with mild insulin resistance and substantial insulin secretion failure; these characteristics are common especially in Japanese individuals because they were unable to bear the unnatural diabetogenic load. Family history of diabetes is a plausible risk factor, but this is also controversial.[22, 27, 34-36] However, at least the diabetes history of first-degree relatives appears to be associated with the development of glucose metabolism disorders.[37] In this report, the first-degree relatives of 4 patients had diabetes or prediabetes, and all of these patients had developed glucose metabolism disorders, which persisted at the follow-up OGTT. There was no statistical significance, but it is reasonable to give careful attention to patients with first-degree family history.

The important point in this study was that our patients underwent the follow-up evaluation when they

were off conventional therapeutic agents. This is the first report about glucose metabolism disorders in patients with refractory NS who have discontinued glucocorticoids and CNIs. These medications could not have been withdrawn prior to demonstrating the effectiveness of rituximab on refractory NS.[6] In this report, 10 patients still had prediabetes 2 years later despite discontinuation of PSL and CNI, indicating that glucose metabolism disorders in refractory NS should not be incautiously considered as reversible conditions. The long-term burden of diabetogenic medications and overworking of pancreatic β cells might cause subsequent progression of irreversible deterioration of pancreatic β-cell function. Specifically, if non-obese/non-overweight patients had glucose metabolism disorders, their insulin secretion capacity might have been impaired remarkably and irreversibly. On the contrary, in some patients, glucose metabolism disorders improved with reduction in weight or discontinuation of PSL and CNI; hence, changing the lifestyle or immunosuppressive regimens remains important.

In this study, 8 of 20 patients with glucose metabolism disorders were detected only by OGTT and 2-hour blood glucose levels. Among them, there were 3 patients with persistent glucose metabolism disorders after the discontinuation of PSL and CNI (Table 3; Patient 4-6). Moreover, we never detected the remarkable impaired insulin secretion in 2 patients (Table 3; Patient 2 & 7) until we performed OGTT, although they had only mild elevation of HbA1c. OGTT is superior in the evaluation of post-load blood

glucose levels and insulin secretory capacity; however, it takes a lot of time and effort, especially in children. On the contrary, fasting blood glucose and/or HbA1c levels are often used for screening diabetes.[15, 16] In this study, 12 cases did not have abnormal HbA1c levels despite having glucose metabolism disorders in OGTT; hence, HbA1c measurement was not sufficient for screening. Fasting blood glucose is also insufficient for screening of steroid-induced diabetes because steroid-induced diabetes is characterized by postprandial hyperglycemia, particularly in the afternoon.[38] Moreover, fasting glucose levels have been reported to be significantly lower in rheumatic patients receiving chronic PSL than in those who had not received oral glucocorticoids for at least 6 months.[39] In this report, blood glucose levels in the first evaluation, during which our patients were receiving PSL, were significantly lower than in the follow-up evaluation, during which our patients had terminated PSL for a median of 15.5 months, suggesting that IFG can be masked during PSL administration. In other words, we may underestimate IFG in patients with PSL administration. Furthermore, in our study, insulinogenic index and AUCins/glu in the follow-up evaluation were lower than in the first evaluation. We speculate that the degradation of insulin secretion was hidden by compensatory action for steroid resistance, which is one of the typical pharmacological actions of a glucocorticoid. Establishment of clearer and more efficient assessment criteria for glucose metabolism disorders in patients with long-term steroid use are

necessary.[5]

Long-term CNI administration appeared to be involved in high frequency of glucose metabolism disorders in our patients. Our patients received a median of >5 years of CNI administration. Calcineurin is distributed widely in various tissues, including the islet cells of pancreas, skeletal muscle, and adipocytes.[40] CNIs are toxic to the islet cells and impair insulin gene expression and insulin secretion.[41] Both tacrolimus and cyclosporine have demonstrated adverse impacts on insulin secretion and sensitivity, tacrolimus being more diabetogenic than cyclosporine.[21, 22] These effects are dose- and duration-dependent and partially reversible with shorter exposures.[42, 43] In our study, the persistence of metabolic glucose disorders after the discontinuation of PSL and CNI also suggests that CNIs' toxicity to islet cells might not be necessarily reversible in patients with long-term CNI administration.

This study had some limitations. This was a cross-sectional study; hence, it was not clear when the patients developed glucose metabolism disorders retrospectively. In addition, the cumulative doses of PSL and CNI had not been evaluated. Another limitation of this study is that we did not measure intra-patient variability.[44] In fact, 3 of 4 patients who had only mild elevation of the 2-hour blood glucose level (140-150 mg/dl) tended to have no glucose metabolism disorders at the follow-up evaluation (Patient 11, 12, & 14). Perhaps these patients should not have been diagnosed as having prediabetes; that

is, the prevalence rate might have been overestimated. A deficiency of data on pubertal status is one more limitation. There was an age discrepancy between the obese/overweight patients and non-obese/non-overweight patients in this study although it was not statistically significant. The higher age might contribute to the higher rate of glucose metabolism disorders in non-obese/non-overweight patients. Although it is not hard to anticipate the development of diabetes in some patients, further long-term follow-up is necessary to demonstrate irreversible glucose metabolism disorders. Moreover, our study was limited by the small sample size and consequently the lack of subanalysis for some confounding factors. Although this study had several limitations as mentioned above and the findings at the follow-up evaluation were only descriptive findings, our result was convincing enough to indicate that glucose metabolism disorders in patients with refractory NS should receive greater attention. At present, there are no published guidelines for when or whom to screen for medication-induced diabetes in pediatric patients with refractory NS, and this paves the way for future studies. Early recognition of children at risk for developing diabetes is necessary to address modifiable risk factors, adjust immunosuppressive regimens, and prevent hyperglycemia and associated morbidity.

Patients with refractory NS are at high risk of developing glucose metabolism disorders, even in childhood. While the need to care for obese/overweight patients has been obvious, our results indicate

that we must also take special care of non-obese/non-overweight patients. The choice of immunosuppressive agents should be individualized based on a patient's glucose metabolism condition, or we might have to try different antidiabetic drugs, whose effect on growth and pubertal development in children remains unknown. Pediatricians and those physicians who treat transited adult patients all need to be aware of the treatment challenges.

Conflict of Interest Statement

The authors declare no conflict of interest.

Ethical approval

All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. This study was approved by the Research Ethics Committee of Hokkaido University Hospital.

Informed consent

Informed consent was obtained from all patients and their parents before study participation.

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Table Legends

Table 1. Patient characteristics at the first evaluation

Data are presented as the median [interquartile range] or n (%)

BMI: body mass index; NS: nephrotic syndrome; PSL: prednisolone; CNI: calcineurin inhibitor; SRNS: steroid-resistant nephrotic syndrome

Table 2. Comparison between obese or overweight and non-obese or overweight

Data are presented as the median [interquartile range] or n (%)

BMI: body mass index; PSL: prednisolone; CyA: cyclosporine A; HOMA IR: Homeostatic Model Assessment of Insulin Resistance; $AUC_{ins/glu.}$ the ratio of the area under the insulin curve to the area under the glucose curve

Table 3. Follow-up evaluation

Age and disease duration are value at the first evaluation. "→" shows "the value or result at the first evaluation → at the follow-up evaluation." PSL washout period means consecutive period without PSL administration just before the follow-up evaluation. Numeric dates in bold are abnormal values. (BMI>85, HbA1c>5.7, Insulinogenic Index<0.4)

BMI: body mass index; HbA1c: hemoglobin A1c; PSL: prednisolone; OGTT: oral glucose tolerance test; HOMA IR: Homeostatic Model Assessment of Insulin Resistance; DM: diabetes; IGT: impaired glucose tolerance; IFT: Impaired fasting glucose; NGT: normal tolerance; n.d.: no date

Table 4. Change of glycometabolic parameters between the first and follow up OGTT

OGTT: oral glucose tolerance test, IQR: interquartile range, HOMA IR: Homeostatic Model Assessment of Insulin Resistance

Figure Legends

Fig.1. Change of the fasting and 2-hour plasma glucose levels between the first and follow-up oral glucose tolerance test

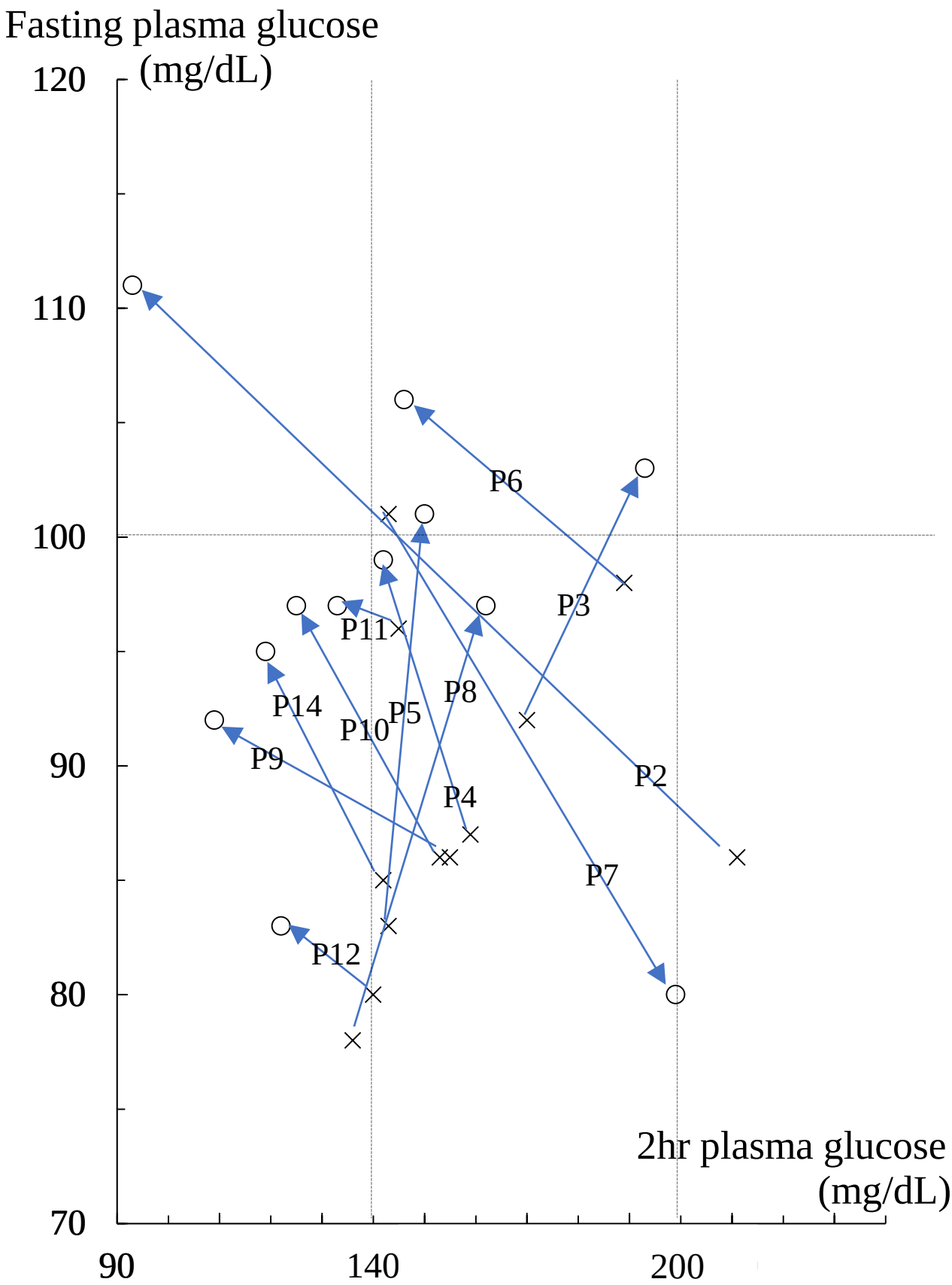
Cross marks at the starting point of each arrow show the fasting and 2-hour plasma glucose levels at the first evaluation, and circle marks at the ending point of each arrow show the fasting and 2-hour plasma glucose levels at the follow-up evaluation. Numbers of each arrow represent the patient numbers in Table 3. Dotted lines show borderlines between normal glucose tolerance and impaired glucose tolerance or impaired fasting glucose and diabetes.

	N=35
Age （years）	12.3[9-13.7]
Sex	
male	21(67%)
female	14(33%)
Height (cm)	135.1[123.8-145.6]
Height for age Z-score（SD）	-1.3[-0.2 - -2.3]
BMI	19.7[17.5-24.7]
BMI percentile	77.3[47.4-97.8]
overweight (>85th and <95th percentile)	5(14%)
obesity (>95th percentile)	10(29%)
Disease duration for NS （months）	87[56-115]
PSL dose at evaluation （mg/kg/day）	0.31[0.11-0.93]
Duration of CNI treatment （months）	70[46-100]
Immunosuppressant for NS at the first evaluation	
mizoribine+cyclosporine	21(60%)
cyclosporine	13(37%)
mizoribine	1(3%)
History of Immunosuppressant use	
cyclosporine	35(100%)
mizoribine	28(80%)
cyclophosphamide	11(31%)
History of mPSL pulse therapy	8(23%)
History of SRNS	14(40%)
First degree diabetes family history	4(11.4%)

	Obese/ overweight (n=15)	Non-obese/ non-overweight (n=20)	p value
Diabetes or Prediabetes	5(33%)	15(75%)	0.019
Sex; male	7(47%)	14(70%)	0.187
First degree family history	0(0%)	4(20%)	0.119
Age (years)	9.8[8.8-12.9]	12.5[10.3-15.0]	0.194
Height (SD)	-2[-0.7 - -4.1]	-0.8[-0.1 - -2.1]	0.069
BMI percentile	98.9[92.8-99]	47.6[18.7-65]	<0.001
Disease duration (months)	83[35-115]	87.5[60.5-114.3]	0.474
PSL (mg/kg/day)	0.34[0.15-0.93]	0.20[0.09-0.96]	0.223
CyA duration (months)	65[30-100]	77.5[58.3-103.3]	0.217
HbA1c (%)	5.6[5.3-5.8]	5.4[5.1-5.7]	0.356
Fasting plasma glucose (mg/dl)	86[85-91]	93.5[86.3-97.5]	0.024
2-hr plasma glucose (mg/dl)	117[105.5-130]	149.5[137.5-158.3]	<0.001
HOMA IR	4.0[2.9-6.2]	2.6[2.0-2.8]	0.003
Insulinogenic index	4.4[3.1-7.4]	2.2[1.1-3.4]	0.002
$AUC_{ins/glu}$	1.30[0.84-1.71	0.72[0.56-1.27]	0.007

	Sex	Age (years)	First degree family history	Disease duration (months)	BMI (percentile)	PSL (mg/kg/day)	PSL Washout period (months)	HbA1c (%)	OGTT	Insulinogenic Index	HOMA IR
Patients with persisting glucose metabolism disorders											
1	F	11.3	-	109	**99.9**→**98.4**	0.34→0	15	**10**→5.5	DM→IGT	n.d.→1.6	n.d.→4.4
2	M	15.1	+	138	47.4→29.4	0.31→0	20	**5.9**→**5.7**	DM→IFG	1.0→**0.3**	2.5→1.8
3	M	13.4	+	102	50.3→45.0	0.43→0	9	**5.7**→5.3	IGT→IFG +IGT	1.2→0.7	2.8→1.4
4	M	7.9	+	62	47.6→31.8	0.10→0	16	5.2→5.4	IGT→IGT	1.5→0.9	1.5→2.5
5	F	10	-	90	65.0→66.9	1.75→0	20	5.4→5.0	IGT→IFG +IGT	3.7→3.7	1.0→4.4
6	M	12.4	-	86	63.9→1.8	1.73→0	9	5.3→5.4	IGT→IFG +IGT	3.2→1.9	2.7→1.3
7	M	11	-	59	79.3→**86.8**	0→0	0	**5.7**→5.6	IFG +IGT→IGT	0.6→**0.1**	2.1→1.2
8	F	8.4	-	35	**99.0**→**99.5**	1.18→0	20	**6.3**→5.4	NGT→IGT	1.0→0.8	2.1→3.7
9	F	12.4	+	63	77.5→33.4	1.26→0	20	5.6→**5.7**	IGT→NGT	2.2→1.4	2.8→0.7
10	M	14.6	-	117	**90.8**→15.0	0.31→0	20	**5.8**→**5.7**	IGT→NGT	9.4→2.9	3.4→2.3
Patients with normalized glucose metabolism											
11	F	15.1	-	117	3.2→44.9	1.14→0	7	4.5→5.6	IGT→NGT	1.1→0.7	2.6→3.7
12	F	9	-	26	**92.5**→72.0	0.81→0	0	5.4→5.4	IGT→NGT	4.0→3.1	3.0→2.1
13	F	8.5	-	13	**97.4**→71.7	0.72→0	3	**6.0**→5.2	n.d.→NGT	n.d.→4.0	n.d.→3.4
14	M	4.4	-	25	75.2→39.1	2.03→0	20	**5.7**→5.6	IGT→NGT	5.0→1.7	3.1→1.3

N=12	the first OGTT			the follow-up OGTT			
	mean±SD	median	IQR	mean±SD	median	IQR	p value
Fasting plasma glucose (mg/dl)	88.2±7.1	86	83.5-95	97±8.8	97	92.8-102.5	0.025
2hr plasma glucose (mg/dl)	158.2±22.6	149	142.3-167.3	141.1±31.8	137.5	119.8-159	0.274
HOMA-IR	2.5±0.7	2.7	2.1-3.0	2.2±1.2	2.0	1.3-3.4	0.519
Insulinogenic index	2.8±2.5	1.8	1.0-4.0	1.5±1.2	1.2	0.7-2.6	0.001
$AUC_{ins/glu}$	1.2±0.8	1.0	0.6-1.7	0.6±0.4	0.5	0.3-1.0	0.001


Fasting plasma glucose
(mg/dL)
120
110
100
90
80
70
90
140
200
2hr plasma glucose
(mg/dL)
P6
P3
P11
P14
P8
P5
P10
P9
P2
P4
P7
P12